

Amendments to the Claims:

Please cancel claims 7 and 13 without prejudice to or disclaimer of the subject matter contained therein. Please amend claims 15 and 91 as follows:

1. (Previously Presented) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with at least one enhancer and an AAV ITR, wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR.
2. (Original) The rAAV vector of claim 1, wherein said rAAV vector further comprises spacer DNA.
3. (Original) The rAAV vector of claim 1, wherein said rAAV is selected from the group consisting of AAV serotype 1, serotype 2, serotype 3, serotype 4, and serotype 5.
4. (Original) The rAAV vector of claim 1, wherein said B-domain deleted factor VIII is a human B-domain deleted factor VIII.
5. (Original) The rAAV vector of claim 4, wherein said heterologous nucleotide sequence encodes a B-domain deleted factor VIII having the amino acid sequence set forth in SEQ ID NO:2.
6. (Original) The rAAV vector of claim 4, wherein said heterologous nucleotide sequence comprises the sequence given as about nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.
7. (Canceled)

8. (Original) A pharmaceutical formulation comprising the rAAV vector of claim 1 in a pharmaceutically acceptable carrier.

9. (Previously Presented) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with a liver-preferred enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR.

10. (Original) The rAAV vector of claim 9, wherein said heterologous nucleotide sequence comprises the sequence given as about nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

11. (Original) The rAAV vector of claim 9, wherein said liver-preferred expression control element comprises at least one enhancer selected from the group consisting of the $\alpha 1$ microglobulin/bikunin enhancer, the hepatitis B virus EnhI enhancer, the hepatitis B virus EnhII enhancer, the human albumin E_{1,7} enhancer, and the human albumin E₆ enhancer.

12. (Previously Presented) The rAAV vector of claim 9, wherein said liver-preferred expression control element comprises the hepatitis B virus EnhI enhancer given as about nucleotides 150-278 of the nucleotide sequence set forth in SEQ ID NO:1.

13. (Canceled)

14. (Original) The rAAV vector of claim 9, wherein said liver-preferred expression control element comprises at least one transcription factor binding site selected from the group consisting of a TATA box, a CAAT box, a GC box, an ATF box, a C/EBP binding site, an HNF1 binding site, an HNF2 binding site, an HNF3 binding site, an HNF4 binding site, and a TGT3 binding site.

15. (Currently amended) The rAAV vector of claim 9, wherein said heterologous nucleotide sequence further comprises sequences encoding ~~a promoter and~~ a polyadenylation sequence.

16. (Original) The rAAV vector of claim 9, wherein said heterologous nucleotide sequence comprises the sequence given as about nucleotides 150 to 4914 of the nucleotide sequence set forth in SEQ ID NO:1.

17. (Original) The rAAV vector of claim 9, wherein said heterologous nucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:2.

18. (Previously Presented) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding a B-domain deleted factor VIII operably linked with an enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and wherein said heterologous nucleotide sequence is selected from the group consisting of:

(a) the nucleotide sequence given as nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1,

(b) a nucleotide sequence that hybridizes to the nucleotide sequence of (a) under conditions of high stringency and which encodes a biologically active B-domain deleted factor VIII, wherein the conditions of high stringency comprise hybridization in 25% formamide and 5X SSC at 42°C and at least one wash in 0.3 M NaCl, 0.03 M sodium citrate at 60°C; and

(c) a nucleotide sequence that that differs from the nucleotide sequences of (a) and (b) above due to the degeneracy of the genetic code, and which encodes a biologically active B-domain deleted factor VIII.

19. (Original) The rAAV vector of claim 18, wherein said rAAV further comprises spacer DNA.

20. (Original) A composition comprising a population of at least about 10^{12} recombinant adeno-associated virus (rAAV) vector particles comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII.

21-57. (Canceled)

58. (Previously Presented) A nucleic acid molecule comprising a nucleotide sequence encoding B-domain deleted factor VIII operably linked with a hepatitis virus enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR.

59. (Previously Presented) The nucleic acid molecule of claim 58, wherein said hepatitis virus enhancer is from a hepatitis B virus.

60. (Previously Presented) The nucleic acid molecule of claim 59, wherein said hepatitis virus enhancer is a hepatitis B virus EnhI or EnhII enhancer.

61. (Previously Presented) The nucleic acid molecule of claim 60, wherein said hepatitis virus enhancer is a hepatitis B virus EnhI enhancer.

62. (Previously Presented) The nucleic acid molecule of claim 58, wherein said nucleic acid molecule comprises the sequence given as about nucleotides 150 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

63. (Previously Presented) The nucleic acid molecule of claim 62, wherein said nucleic acid molecule further comprises a polyadenylation sequence.

64. (Previously Presented) The nucleic acid molecule of claim 63, wherein said nucleic acid molecule comprises the sequence given as nucleotides 150 to 4914 of the nucleotide sequence set forth in SEQ ID NO:1.

65. (Previously Presented) A vector comprising the nucleic acid molecule of claim 58.

66. (Original) The vector of claim 65, wherein said vector is the plasmid disclosed herein as pDLZ6.

67. (Original) A cell containing the vector of claim 65.

68. (Previously Presented) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence operably linked with an enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and wherein said heterologous nucleotide sequence is at least 75% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

69. (Previously Presented) The recombinant adeno-associated virus (rAAV) vector of claim 68, wherein said heterologous nucleotide sequence is at least 80% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

70. (Previously Presented) The recombinant adeno-associated virus (rAAV) vector of claim 69, wherein said heterologous nucleotide sequence is at least 85% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

71. (Previously Presented) The recombinant adeno-associated virus (rAAV) vector of claim 70, wherein said heterologous nucleotide sequence is at least 90% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

72. (Previously Presented) The recombinant adeno-associated virus (rAAV) vector of claim 71, wherein said heterologous nucleotide sequence is at least 95% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

73. (Previously Presented) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence operably linked with a hepatitis virus enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and wherein said heterologous nucleotide sequence is at least 75% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

74. (Previously Presented) The rAAV vector of claim 73, wherein said hepatitis virus enhancer is from hepatitis B virus.

75. (Previously Presented) The rAAV vector of claim 74, wherein said hepatitis virus enhancer is a hepatitis B virus EnhI or EnhII enhancer.

76. (Previously Presented) The rAAV vector of claim 75, wherein said rAAV vector comprises the sequence given as about nucleotides 150 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

77. (Previously Presented) The rAAV vector of claim 73, wherein said rAAV vector further comprises a polyadenylation sequence.

78. (Canceled)

79. (Previously Presented) A cell comprising the rAAV vector of claim 77.

80. (Previously Presented) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence operably linked with an enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and wherein said heterologous nucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:2.

81. (Previously Presented) The recombinant adeno-associated virus (rAAV) vector of claim 80, wherein said heterologous nucleotide sequence is the nucleotide sequence given as nucleotide 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

82. (Previously Presented) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence operably linked with at least one enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and wherein said heterologous nucleotide sequence is at least 75% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

83. (Previously Presented) The recombinant adeno-associated virus (rAAV) vector of claim 82, wherein said heterologous nucleotide sequence is at least 80% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

84. (Previously Presented) The recombinant adeno-associated virus (rAAV) vector of claim 83, wherein said heterologous nucleotide sequence is at least 85% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

85. (Previously Presented) The recombinant adeno-associated virus (rAAV) vector of claim 84, wherein said heterologous nucleotide sequence is at least 90% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

86. (Previously Presented) The recombinant adeno-associated virus (rAAV) vector of claim 85, wherein said heterologous nucleotide sequence is at least 95% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

87. (Previously Presented) The rAAV vector of claim 82, wherein said vector further comprises spacer DNA.

88. (Previously Presented) The rAAV vector of claim 82, wherein said rAAV is selected from the group consisting of AAV serotype 1, serotype 2, serotype 3, serotype 4, and serotype 5.

89. (Canceled)

90. (Previously Presented) A pharmaceutical formulation comprising the rAAV vector of claim 82 in a pharmaceutically acceptable carrier.

91. (Currently Amended) The rAAV vector ~~method~~ of claim 1, wherein said rAAV vector is capable of expressing B-domain deleted factor VIII at a level sufficient for treatment of a factor VIII associated-disorder.

92. (Previously Presented) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with at least one enhancer and an AAV ITR, wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV and said rAAV vector is capable of

Appl. No.: 09/689,430

Filed: Walsh *et al.*

Page 10

expressing B-domain deleted factor VIII at a level sufficient for treatment of a factor VIII associated-disorder.